

EFFECT OF THE ANTIOXIDANT ACTION OF GINKGO BILOBA EXTRACT (EGb 761) ON AGING AND OXIDATIVE STRESS

M. T. Droy-Lefaix

Institut IPSEN

24 rue Erlanger

75016 Paris, France

TEL: 33.Ø1.44.96.10.10

FAX : 33.Ø1.44.96.11.99

ABSTRACT

Aging is responsible for oxidative damage to DNA, protein, lipid, and other macromolecules linked to tissue alterations. The resultant damage contributes significantly to degenerative diseases, to include those of the brain, sensorial tissues, and cardiovascular system. To protect cellular components from oxyradical attack, especially lipoperoxidation, a substantial interest in the use of antioxidants has evolved. A free radical scavenger, Ginkgo biloba extract (EGb 761) may be effective in fighting the oxidative stress related to aging. Many data support the efficacy of EGb 761 in biological model systems. In aging processes, EGb 761 may ameliorate the mitochondria respiratory chain function by quenching the superoxide anion, and the hydroxyl and peroxyl radicals. It protects the brain by facilitating the uptake of neurotransmitters and by reducing ischemia-reperfusion episodes and level of apoptosis. Moreover, in sensorial tissues, EGb 761 reduces apoptosis in the olfactory bulb and in the retinal pigmented epithelium of the eye, and protects against the lipoperoxidation alteration of the retina that results in a decrease of the electroretinogram response. In the cardiovascular system, by a direct effect on oxidative low density lipoproteins, EGb 761 may decrease atherosclerosis evolution, and is shown to accelerate cardiac mechanical recovery after ischemia-reperfusion. In conclusion, the antioxidant effects of EGb 761 noted in many experimental data, may explain the therapeutic efficacy observed in clinical trials of the elderly. These beneficial properties seem in part to come from the activity of EGb 761 constituents, such as flavonoids and terpens.

KEY WORDS

Ginkgo biloba extract, Antioxidants, Oxidative stress, Aging

INTRODUCTION

Over the past several years, research has shown reactive free radicals ($O_2^{\cdot-}$, OH^{\cdot} , ROO^{\cdot}) have a role in the general processes of aging and tissue damage (1-4). Oxidative stress can influence the evolution of many

degenerative diseases including those involved with the cardiovascular system, brain and eye dysfunction, and carcinogen metabolism (1,5). Growing evidence shows that over the human life span, some oxidative attack can be prevented by the presence of endogenous antioxidants. Many defense mechanisms are involved in the limiting of free radical damage: enzymes such as superoxide dismutase; catalase and glutathione peroxidase; free iron or copper chelators (1); and molecular antioxidants such as ascorbic acid (vitamin C) and α -tocopherol (vitamin E) (6-9).

Each component of this antioxidant defense system may combine synergistically to protect the organism. However, a deficiency in their response from a strong increase in free radical activity, may cause a loss of efficacy and be responsible for tissue damage and functional disturbances (10).

To protect cellular components from free radical damage, there is substantial interest in scavenging the various forms of active oxygen, especially the hydroxyl radical (OH^{\cdot}), which is considered responsible for most of the peroxidative degradation linked to the structural disorganization of membranes (8). Natural substances such as Ginkgo biloba extract (EGb 761) (IPSEN, France) can be an effective compound in protecting against oxidative stress and aging.

Background on EGb 761

Ginkgo biloba is a tall, sturdy, extremely long-lived tree with leaves divided into two lobes (11). Extract from the leaves of Ginkgo biloba tree (EGb 761) was introduced in Europe in 1965 into medical practice for treatment of peripheral arterial diseases and cerebral insufficiency in the elderly (13,14). EGb 761 is now used in a broad spectrum of pharmacological actions; it was demonstrated that EGb 761 can be required for anti-ischemic, antiedema, antihypoxic, radical scavenging, metabolic actions, as well as for rheology improvements. Beneficial clinical interactions have been noted in the brain, heart, eye and ear (15,16).

Constituents of EGb 761 appear to contribute to its pharmacological activity (13). EGb 761 is made from dried leaves using highly standardized procedures. This extract is a complex mixture containing 24% flavonoid glycosides (ginkgoflavones, glycosides), 6% terpen lactones, such as ginkgolides A, B, C, J and bilobalide, some organic acids, and other constituents

(13). Of the EGb 761 chemical constituents, the flavonoids, by their ability to scavenge the free radicals, have important therapeutic applications. The ginkgo flavone glycosides are mono-, di- or triglycosides whose aglycon is a flavonol (quercetin, kaempferol or isorhamnetin) and whose glycosidic constituents are glucose and rhamnose (17).

Antioxidant Action of EGb 761

Effect on superoxide anion. Superoxide anion ($O_2^{\cdot-}$), made by adding one electron to the oxygen molecule, is formed in vivo in a variety of ways, such as through the activities of the mitochondria electron transport chain, the arachidonic chain, the xanthine oxidase system, and by phagocytes such as neutrophils. The generation of superoxide contributes to the conversion into hydroxyl radical (OH^{\cdot}) through hydrogen peroxide (H_2O_2) in the presence of a metal ion, such as copper or iron (5,18). As demonstrated by in vitro experiments, EGb 761 may scavenge $O_2^{\cdot-}$ when generated by irradiation with gamma rays (19,20) or by phenazine methosulfate-NADH (16). Moreover, EGb 761 can inhibit xanthine oxidase activity in a dose-dependent manner that interferes with $O_2^{\cdot-}$ formation (20).

Effect on hydroxyl radical. Some scavenger effects were noted with EGb 761 on the hydroxyl radical, OH^{\cdot} . This radical is extremely reactive and toxic to cell membranes. EGb 761 is shown to scavenge hydroxyl radicals in a dose-dependent manner, as demonstrated in the model using dihydroxybenzoic acid on the Fenton reaction (21) or using gamma rays (19). The scavenging activities of EGb 761 seem correlated with the presence of flavonoids in the extract (22-25).

Effects on lipoperoxides. Similarly, EGb 761 in rat liver microsomes may inhibit the peroxidizing system, induced by a $FeCl_3$ /ADP/NADPH mixture (21). EGb 761 is also effective in protecting against the formation of malondialdehyde induced by UVC irradiation, linked to polyunsaturated fatty acid damage (26). In a dose-dependent manner, EGb 761 also quenches the peroxy radicals generated by the azo initiator, 2,2'-azobis (2,4-dimethylvaleronitrile) (AMVN), as detected in liposomes in the presence of DOPC by the production of chemiluminescence (27). This EGb 761 effect on the peroxide formation may be due to the presence of flavonoids in the extract (24,28).

Effect on nitric oxide. EGb 761 may also scavenge nitric oxide in a dose-responsive manner by a reaction of hydroxylamine with complex I of the catalase. EGb 761 also inhibits the nitric oxide induced by the hemoglobin oxidation; furthermore, when nitric oxide is generated from sodium nitroprusside, the extract limits the accumulation of nitrite generated during the reaction of nitric oxide with oxygen. This EGb 761 effect may be the consequence of the EGb 761 reaction with the other oxides of nitrogen (NO_2 , N_2O_3 , N_2O_4) and the peroxy nitrite ($OOONO^{\cdot}$), which seem possible intermediates in the oxidation of nitric oxide to nitrite (29).

Aging and the Biological Approach of EGb 761

As demonstrated by Harman in 1956 (30), experimental evidence supports the notion that oxidative components have an important place in aging processes in that their increases during senescence create a tissue imbalance in favor of the oxidants. In relation to the aging phenomenon, as free radicals appear involved in various cellular functions, EGb 761 antioxidant effects were tested on mitochondria function, cellular senescence, and on brain, eyes, and cardiovascular aging disorders.

EGb 761, mitochondria, and aging. Increased generation of oxygenated free radicals may be responsible for the age-associated oxidative damage that occurs in mitochondria (31). Mutations of mitochondrial DNA linked to a decline in the respiratory chain have been reported in liver (32,33), brain (34), and skeletal muscle (35).

EGb 761 was demonstrated to protect cardiomyocyte mitochondria in elderly rats (17 mo) placed under hypoxia by the controlled application of an O_2 - N_2O mixture with low O_2 content of 5 vol % within 5 min. This condition was maintained for 13 min before the O_2 level was down-regulated to 0 within 2 min. The effect of hypoxia was observed in the internal mitochondria using ultrastructural morphometry. In EGb 761 treated rats, at the dose of 100 mg/kg given orally 3 mo before exposure to hypoxia, the reduction of the surface density cristae and the degeneration of intramitochondrial areas greatly differed from the untreated rats. This EGb 761 protective effect on hypoxia-related lesions can be interpreted as a membrane stabilizing action, based on the radical scavenging properties of the extract (36).

Prevention of age-associated oxidative stress in mitochondria (mt) was confirmed measuring by the amount of oxidized base, 8 oxo-7-8 dihydro 2'-deoxyguanosine (oxo 8 dG), as a biomarker of oxidative DNA. In brain and liver of old rats (27 mo) compared to young rats (4 mo), the oxidative damage to mtDNA increased with the age. EGb 761 treatment, given orally at the dose of 100 mg/kg during 3 mo before the mtDNA evaluation, significantly prevented mtDNA damage (37).

In addition, in the same experiment, peroxide generation measurements in young and old rats confirmed that the mtDNA protective effect of EGb 761 was due to the antioxidant properties of the extract. The lipoperoxide release was prevented by EGb 761 treatment (37). Furthermore, the oxidative damage to the mtDNA that occurs during aging, seemed related to the oxidation of mitochondrial glutathione and the increase of the GSSG/GSH ratio noted in untreated rats; these results confirm the key role of mitochondrial glutathione in the protection against free radical damage (37). Treatment with EGb 761 prevented the age-related increase of GSSG at the origin of an increase in the GSSG-GSH ratio.

The administration of antioxidants, such as EGb 761, has therefore been successful in protecting mitochondrial function. Age-related changes are well-demonstrated to interfere with the mitochondrial membrane potential linked to respiratory activity (35). Modifications

in respiratory parameters were determined, especially in state 4 (the resting mitochondria respiration rate), measured in the absence of added ADP. In state 4 respiration, the stimulation of the membrane potential in brain mitochondria from 27-month-old rats was around 30% of that found in state 4 of young rats. Treatment with EGb 761 (100 mg/kg/po/3mo) was effective in preventing a decrease in energy status (37). Similar results were noted in mitochondrial stage 3 respiration of aged rats, corresponding to phosphorylation respiration. Old Fischer rats (18 mo), given 50 mg/kg for 14 days, showed a significant increase in oxygen consumption (+17%) by mitochondria compared to untreated rats (38).

All these data confirm that oxidative stress is a major contributor to the mitochondrial impairment noted with aging. EGb 761 may be helpful in preventing mitochondrial disorders caused by free radical generation during aging.

Aging and brain disorders

Neurotransmitters. The brain is extremely sensitive to the presence of the oxygenated free radicals that directly interfere with the polyunsaturated fatty acids (PUFA) in the membrane, resulting in changes in the membrane structures associated with neurotransmitter uptake disorders (39,40). EGb 761 can totally inhibit the PUFA degradation and the appearance of thiobarbituric acid-reactive substances (TBARS) in the microsomes submitted to UVC irradiation (41). Thus, EGb 761 may provide effective and persistent protection to the membrane and thereby to the neurotransmitter uptake, as demonstrated in synaptosomal fractions prepared from mouse cerebral cortex. When EGb 761 was directly added to these fractions, there was an increase of serotonin uptake (42). A similar effect was observed when the synaptosomes were prepared from the cortex of mice treated orally with EGb 761 (100 mg/kg, 14 hr and 2 hr before the sacrifice). After treatment with EGb 761, synaptosomal uptakes of dopamine and serotonin were maintained.

Different hypotheses have been formulated to account for the changes in nervous system functions during aging processes. Because the functional activity of the nervous system is largely governed by neurotransmitter receptors, the influence of aging was tested on alpha 2-adrenoreceptors and 5HT1A receptors in rat hippocampus and the cerebral cortex, which are cognitive brain areas (43). In 24-month-old rats, the specific ($_3$ H)8-OH-DPAT binding to 5HT1A receptors was lower in cerebral cortex membranes compared to young, 4-month-old animals. A scatchard analysis showed a significant decrease in the number of ($_3$ H)8-OH-DPAT binding sites in the senescent rats. When the rats were treated with EGb 761 (5mg/ip/21d) before measurements, there was a significant increase in the binding of ($_3$ H)8-OH-DPAT compared to untreated animals (43). The same results were noted in the alpha-2 adreno-

receptors, especially in the hippocampus. In aged rats, a significant increase of ($_3$ H) rauwolscine binding to hippocampus occurred in the presence of EGb 761 (5mg/ip/21d). These data confirm that EGb 761 may block the auto-oxidative membrane process often linked to a decrease in fluidity and an increase in viscosity of the neuronal membranes (44), which is known to induce subsequent behavioral changes (45).

Peroxidation levels and antioxidant enzymes. During aging processes, lipid peroxidation is shown to increase in the cortex and the hypothalamus, as measured in 24-month-old rats. EGb 761, given orally at doses of 50 and 100 mg/kg for 12 weeks significantly decreased the level of lipid peroxidation in these two areas. Superoxide dismutase levels in liver were also significantly increased when the rats were treated with EGb 761 at the same doses (46). Similar data were noted in 33-month-old rats. In such experiments, EGb 761 given orally at a dose of 100 mg/kg/day for 3 mo significantly increased rat survival and decreased the level of peroxide generation in the brain, as noted by TBARS measurements (47).

Cerebral ischemic damage. During aging, ischemic injury might be thought as an essential cause of the partial disruption in brain tissue at the origin of free radical reactions, especially at the moment of the reperfusion (48,49). The administration of EGb 761, given orally or intraperitoneally to gerbils prevented the cerebral edema and the impairment of stroke index due to cerebral ischemia (50). According to these data, therapeutic intervention with antioxidants that cross the blood-brain barrier might be of value in the protection against brain tissue injury.

Apoptosis

Apoptosis is a form of programmed cell death controlled by an elaborate network of signaling pathways through receptors, protein kinases, second messengers, phosphorylated protein intermediates, and factors that can regulate the expression of individual genes or groups of genes (51). Apoptosis has been proposed as the final event in the existence of many cells within a multicellular organism (52), and its role may be important to the life span of individual cells. Apoptosis is actually well-demonstrated to be associated with aging diseases that have a reduced immune response (53). With appropriate activation, apoptotic cell death may be responsible for the neuronal loss typical in neurodegenerative diseases. As exposure to oxidants (54) can induce multiple cellular interactions, using antioxidants can provide a general protection against different types of apoptosis (55). In this way, sensorial neurons such as the olfactory neurons, are known to be more sensitive to apoptotic effects than central neurons by reason of their frequent exposure to aggressive factors, physical, chemical, or biological, vehiculed by inspired air. Olfactory neurons, therefore, seem a good target to study apoptosis mecha-

nisms in the nervous system. An experimental model, characterized by axotomy, can induce apoptosis in rats, with DNA degradation, 24 to 48 hr following lesion formation. In such a model, apoptosis was quantified using autoradiographic detection of internucleosomal fragmentation of labeled DNA. When the rats were pretreated with EGb 761, given orally 10 days at the dose of 50 mg/kg, there was a clear trend towards the reduction of the DNA fragmentation. The conclusion derived from these results is that EGb 761 has a protective effect against apoptosis of primary olfactory neurons and supports the fact that free radical generation is involved in the apoptosis process (56).

Aging and retina

Over the past several years, the toxic effect of oxygen species have been evaluated in the eye, especially the retina, which is very sensitive to free radical disorders. In fact, the vertebrate retina consumes 5-10 times more oxygen per mg than other tissues; and the photoreceptors, the keys of vision, contain high concentrations of polyunsaturated fatty acids, and therefore are very sensitive to oxidant attack. As with the other tissues, there is fairly convincing evidence that free radicals are involved in degenerative, aging eye diseases. In some vascular accidents, such as ischemic disorders, apoptosis may be associated with aging and be responsible for functional degeneration of the retina and visual response.

Retina ischemia disorders. In ischemia-reperfusion, oxygenated free radicals may be generated by several pathways including the xanthine-oxidase system (the NADPH-dependent oxidase of neutrophils), the mitochondria electron transport chain, and arachidonic metabolism (57). To verify the effect of the antioxidants on the retina, EGb 761 was given 10 days before the experiment to albino Sprague-Dawley rats. The central retinal artery was occluded by clamping 90 min, then reperfusion was permitted at 4-24 hr. The rats were then sacrificed and the retinas were released for analysis. In untreated rats, histological retina analysis showed degenerative changes with important edema and a neutrophil invasion into the different layers of the retina. With EGb 761 pretreatment, the free radical detrimental effects were significantly reduced (58,59).

Because free radicals can induce histological injury, alterations in the redox state, such as thiols or disulfide bonds, can modify membrane permeability by a variety of ions, such as Na^+ , K^+ , Mg^{2+} , Ca^{2+} (60). In the same ischemia-reperfusion model using occlusion, retina ion contents were measured by atomic absorption spectrophotometry using various wavelengths after washing-out the blood and extracellular fluid, then drying and ashing the retina. In untreated rats, retina vulnerability to ion homeostasis was noted with an increase in Na^+ and Ca^{2+} ions and a decrease of K^+ and Mg^{2+} ions. Pretreatment with EGb 761 (100mg/kg/po) administered 10 days before the experiment and until the

animals were sacrificed, significantly reduced the ion shift imbalance that could be responsible for functional disturbances (60).

To verify the ischemia-reperfusion consequences on visual function, a model of ischemia-reperfusion by hypertony was developed in Fischer rats. Under chloral hydrate anesthesia, the ischemia-reperfusion was carried out on one eye under local anesthesia of cornea using oxybuprocaine, with the other eye serving as control. Ischemia was induced with a gauge needle (0.3 mm diameter), directly introduced into the anterior chamber and linked to an NaCl (0.9 %) bottle situated 1.50 m above the eye to maintain a 110 mmHg pressure. After 1 hr of ischemia, the needle was removed and the reperfusion was permitted. At the dose of 100 mg/kg, EGb 761 was given orally 15 days before the experiment. To analyze the retina functional disorders, an electroretinogram (ERG) was recorded under stimulation of white light flashes (20 ms, 1200 lux) every 10 sec. The b-wave ERG amplitudes of the two eyes, one control and one ischemic were analyzed and recorded before ischemia, and 1, 4, 24 and 48 hr after reperfusion. In treated rats, the results showed that the b-wave ratio, comparing the ratio between the surface on the ischemic and the non ischemic eye, was significantly higher compared to untreated rats, which indicated a decrease in the deleterious effects of the ischemia-reperfusion. Scanning electron microscopy of retina samples, released and fixed 48 hr after the reperfusion, confirmed these electrophysiology observations. In ischemic retina, the retina layers, especially the photoreceptor layer, were totally disorganized with a junction rupture between the inner and outer segment, and showing necrosis and edema. After EGb 761 pretreatment, the retina kept a normal aspect, with disappearance of degenerative lesions (61,62).

Aging and retina degeneration. In sensorial tissues, functional performances decrease with age, in major part, from light exposure. Repeated photon fluxes can induce, by energy transfer, the water molecule lysis responsible for hydroxyl radical (OH^\bullet) formation. This OH^\bullet release is at the origin of the lipoperoxidation-inducing lesions and functional disturbances of the retina membrane. EGb 761 was tested in aged rats by measuring the cumulative effect of white light on the retina, using the b-wave ERG amplitude variation in isolated retina.

To appreciate the age influence, ERG signals in 10- and 20-month-old albino rats, induced by white light flashes (300 lux, 1 ms) every 5 min, were compared with those of 4- to 6-month-old rats. In 10-month-old rats, the b-wave retina survival curve maintained a normal shape, but the amplitude of this curve decreased from a value of 350 μV to 120-140 μV . In 20-month-old rats, the alteration of the curve was marked, and the decrease in the amplitude of the b-wave was at a level of 80-100 μV . Morphological disorders accompanied functional alterations.

When the rats were pretreated 3 months before the b-wave registration with EGb 761 (100 mg/kg/po), the decrease in the b-wave amplitude and the histological damage was not so marked as in the untreated rats, indicating that the use of antioxidants can prevent the retina degenerative processes by scavenging free radicals.

Retinal pigmented epithelial and apoptosis. In the retinal pigmented epithelial (RPE), peroxynitrite toxicity could be mediated at least in part by apoptosis. In specific redox environment, nitric oxide (NO) could combine with the superoxide anion to generate peroxynitrite (ONOO⁻), an even more potent toxic species (63).

In RPE, the extent of cell apoptosis was analyzed using the TUNEL assay. Cells isolated from bovine were treated with 1.25 or 2.5 mM peroxynitrite and DNA fragmentation was determined at 3 and 6 hr after treatment. A simple observation of the nuclei, stained with DAPI, suggests apoptosis 6 hr after treatment, showing fragmented, condensed nuclei and apoptotic bodies.

EGb 761, a scavenger of NO, potentially protected the RPE cells from its antiproliferative action and partially against peroxynitrite-induced cell death, suggesting that EGb 761 could be also a peroxynitrite scavenger (64).

Vascular disease and aging

LDL oxidation. Aging slowly brings on many blood vessel changes, such as hardening of the arteries or arteriosclerosis. Among the factors that may influence the progress of arteriosclerosis and development of fatty plaques in rats, elevated levels of low density lipoproteins (LDL) cholesterol take a major place (65). Because oxidative modification of LDL depends on the oxygenated free radical process, the EGb 761 effect was studied (27,66). EGb 761 prevented lipid peroxidation and the loss of both vitamin E and beta carotene in human LDL oxidation initiated by gamma-ray irradiation, according to a dose-response effect (66). Another model exhibited similar results, using two other initiating systems of LDL oxidation, the hydrophilic peroxy radical initiator 2,2'-azobis (2-amidinopropane) hydrochloride (AAPH) and the hydrophobic initiator, AMVN. EGb 761, in a dose-response manner, prevented lipid peroxidation and loss of both vitamin E and B carotene, completely inhibiting LDL oxidation at the concentration of 100 µg/ml (27).

In addition, the protective effects of EGb 761 against peroxidation of LDL protein were tested by observing a decrease of tryptophan fluorescence induced by incubation with AAPH. EGb 761 (100 µg/ml), completely suppressed the decrease in fluorescence intensity associated with LDL oxidation (27). These data show that EGb 761 may have a protective effect on LDL protein oxidation and thereby can preserve cells from arteriosclerosis evolution.

Cardiac ischemia-reperfusion. During ischemia-reperfusion of the heart, oxygen-derived free radicals play an important role in the genesis of tissue injury (67). Much data confirm that superoxide anion, hydrogen peroxide, and hydroxyl radicals are involved in the reperfusion injury of the post-ischemic heart (68,69).

EGb 761 may modulate the cardiac mechanical recovery during the 20 min period of reperfusion following the 40 min ischemia, as demonstrated in the Langendorff perfused rat heart. The addition of EGb 761 (200 mg/l) to the perfusion medium of the hearts, induced a significant improvement of the recovery (70).

Effects of cardiac ischemia-reperfusion on myocardial ascorbate were also noted. Without EGb 761, total ascorbate content and ascorbate (reduced form) were significantly decreased after 20 min of reperfusion, suggesting that it acted as an antioxidant. In the presence of EGb 761 (200 mg/ml), ascorbate content stayed within normal limits (70).

These results show that EGb 761 prevents ascorbate leakage and oxidation. These data indicate that EGb 761 may react directly with free radicals and its cardiac protective effect may depend on its free radical quenching properties.

SUMMARY

Aging is a multifactorial process in which oxygenated free radicals from exogenous and endogenous sources are involved. One of the most destructive processes is lipoperoxidation, the origin of a chain reaction, which induces DNA, protein, and lipid degradation, causing cell disorders.

The consequences of these free radical oxidant effects are functional disturbances associated in elderly with such neurodegenerative diseases as Parkinson's and Alzheimer's (71), vision disorders, and cardiovascular diseases such as atherosclerosis (71). Using antioxidants may attenuate the deterioration of the physiological properties associated with aging; experimental evidence shows that the extract of Ginkgo biloba (EGb 761), a free radical scavenger, may protect against the aging phenomenon. As demonstrated by several data, EGb 761 may decrease functional alterations, including ischemia-reperfusion, especially in the brain, sensorial tissues, and cardiovascular system. The anti-aging protective effects of EGb 761 have been observed in various experimental models, including the brain, eye, and heart. Furthermore, the results obtained with EGb 761 treatment may explain the therapeutic efficacy noted in some clinical trials. EGb 761, shown by electron-spin-resonance, can reduce oxyradical formation in oxygen-deprived myocardium, as was noted during surgery for coronary artery disease. It also facilitates myocardial function recovery at reperfusion (72). Similarly, EGb 761 may be beneficial to cerebral insufficiency (14,73). It can improve the cognitive function (74,75), and ameliorate patients suffering from psychorganic syndrome (76). Its efficacy has also been proven

in patients suffering from mild to moderate degrees of either multi-infarct dementia or primary degenerative dementia of the Alzheimer type (77). In conclusion, the antioxidant properties of EGb 761 can lead to new and novel approaches to therapy in elderly oxidant situations.

REFERENCES

- Ames, B.N., Shigenaga, M.K. and Hagen, T.M.: Oxidants, antioxidants and the degenerative diseases of aging. *Proc. Natl. Acad. Sci.*, 90: 7915-7922, 1993.
- de Groot, H.: Reactive oxygen species in tissue injury. *Hepato-Gastroenterol.*, 41: 328-332, 1994.
- Dianzani, M.V.: Free radicals in physiology and pathology. *Boll. Soc. It. Biol. Sper.*, LXVIII: 491-511, 1992.
- Zimmerman, J.J.: Oxyradical pathophysiology. *Adv. Pediat.*, 42: 243-302, 1995.
- Halliwell, B.: Reactive oxygen species and the central nervous system. *J. Neurochem.*, 59: 1609-1623, 1992.
- Barber, D.A. and Harris, S.R.: Oxygen free radicals and antioxidants: a review. *Amer. Pharm.*, NS34: 26-35, 1994.
- Beyer, R.E.: The role of ascorbate in antioxidant protection of biomembranes: interaction with vitamin E and coenzyme Q. *J. Bioenerg. Biomembrane*, 26: 349-358, 1994.
- Halliwell, B.: Free radicals, antioxidants and human disease: curiosity, cause, or consequence? *Lancet*, 344: 721-724, 1994.
- Landvik, S.V., Diplock, A.J. and Packer, L.: Efficacy of vitamin E in human health and disease, in *Handbook of Antioxidants*, edited by Cadenas, H. and Packer, L., New York, Marcel Dekker Inc., 1996, pp. 63-87.
- Barja de Quiroga, G., Lopez-Torres, M. and Perez-Campo, R.: Relationship between antioxidants, lipid peroxidation and aging, in *Free Radicals and Aging*, edited by Emerit, I. and Chance, B., Basel, Birkhäuser Verlag, 1992, pp. 109-123.
- Michel, P.F.: *Ginkgo biloba: l'arbre qui a vaincu le temps*. Paris, Editions du Félin, 1986.
- Chang, H.M., and But, P.P.H.: *Pharmacology and applications of Chinese materia medica*. World Scientific Publ. Co, Singapore, 2: 1096-1101, 1987.
- DeFeudis, F.V.: *Ginkgo biloba extract (EGb 761): Pharmacological activities and clinical applications*. Paris, Elsevier, 1991.
- Kleijnen, J. and Knipschild, P.: *Ginkgo biloba*. *Lancet*, 340: 1136-1139, 1992.
- Droy-Lefaix, M.T., Baudouin, C., de Kozak, Y. and Doly, M.: Free radicals and antioxidants in pathophysiology of mammalian retina, in *Free Radicals in Brain Physiology and Disorders*, edited by Packer, P., Hiramatsu, M., and Yoshikawa, T., San Diego, Academic Press, 1996, pp. 359-367.
- Haramaki, N., Packer, L., Droy-Lefaix, M.T., and Christen, Y.: Antioxidant actions and health implications of Ginkgo biloba extract, in *Handbook of Antioxidants*, edited by Cadenas, E., and Packer, L., New York, Marcel Dekker Inc., 1996, pp. 487-510.
- Vanhaelen, M., and Vanhaelen-Fastré, R.: Countercurrent chromatography for isolation of flavonol glycosides from Ginkgo biloba leaves. *J. Liq. Chromatogr.* 11, 2969-2975, 1988.
- Halliwell, B., and Gutteridge, J.M.C.: *Free radicals in biology and medicine*. Oxford, Clarendon Press, 1989.
- Gardès-Albert, M., Ferradini, C., Sekaki, A., and Droy-Lefaix, M.T.: Oxygen-centered free radicals and their interaction with EGb 761 or CP202, in *Advances in Ginkgo biloba extract research*, Vol. 2, *Ginkgo biloba extract (EGb 761) as a free-radical scavenger*, edited by Ferradini, C., Droy-Lefaix, M.T., and Christen, Y., Paris, Elsevier, 1993, pp. 1-11.
- Marcocci, L., Packer, L., Droy-Lefaix, M.T., Sekaki, A.H., and Gardès-Albert, M.: Antioxidant action of Ginkgo biloba extract EGb 761, in *Methods in Enzymology*, edited by Packer, L., San Diego, Academic Press, 1994, pp. 462-475.
- Pincemail, J., and Deby, C.: Propriétés antiradicalaires de l'extrait de Ginkgo biloba. *Presse Méd.*, 15: 1475-1479, 1986.
- Robak, J., and Gryglewski, R.J.: Flavonoids are scavengers of superoxide anions. *Biochem. Pharmacol.*, 37: 837-841, 1988.
- Husain, S.R., Cillard, J., and Cillard, P.: Hydroxyl radical scavenging activity of flavonoids. *Phytochemistry*, 26: 2489-2491, 1987.
- Bors, W., Heller, W., Michel, C., and Saran, M.: Flavonoids as antioxidants: determination of radical-scavenging efficiencies. *Methods Enzymol.*, 189: 343-355, 1990.
- Sichel, G., Corsaro, C., Scalia, M., Di Bilio, A.G., and Bonomo, R.P.: In vitro scavenger activity of some flavonoids and melamino against O₂·-. *Free Rad. Biol. Med.*, 11: 1-8, 1991.
- Dumont, E., Petit, E., Tarrade, T., and Nouvelot, A.: UV-C irradiation-induced peroxidative degradation of microsomal fatty acid and proteins: protection by an extract of Ginkgo biloba (EGb 761). *Free Rad. Biol. Med.*, 13: 197-203, 1992.

27. Maitra, I., Marcocci, L., Droy-Lefaix, M.T., and Packer, L.: Peroxyl radical scavenging activity of Ginkgo biloba extract EGb 761. *Biochem. Pharmacol.*, 49: 1649-1665, 1995.
28. Yuting, C., Rongliang, Z., Zhongjian, J., and Yong, J.: Flavonoids as superoxide scavengers and anti-oxidants. *Free Rad. Biol. Med.*, 9: 19-21, 1990.
29. Marcocci, L., Maguire, J.J., Droy-Lefaix, M.T., and Packer, L.: The nitric oxide-scavenging properties of Ginkgo biloba extract, EGb 761. *Biochem. Biophys. Res. Comm.*, 201: 748-755, 1994.
30. Harman, D.: Aging : a theory based on free radical and radiation chemistry. *J. Gerontol.* 11: 298-300, 1956.
31. Benzi, G., and Moretti, A.: Age- and peroxidative stress-related modifications of the cerebral enzymatic activities linked to mitochondria and the glutathione system. *Free Rad. Biol. Med.*, 19: 77-101, 1995.
32. Wallace, D.C.: Disease of the mitochondrial DNA. *Ann. Rev. Biochem.*, 61: 1175-1212, 1992.
33. Wallace, D.C.: Mitochondrial genetics : a paradigm for aging and degenerative diseases? *Science*, 256: 628-632, 1992.
34. Bowling, A.C., Mutisya, E.M., Walker, L.C., Price, D.L., Cork, L.C., and Beal, M.F.: Age-dependent impairment of mitochondrial function in primate brain. *J. Neurochem.*, 60: 1964-1967, 1993.
35. Trounce, I., Byr, N.E., and Marzuki S.: Decline in skeletal muscle mitochondrial respiratory chain function: possible factor in aging. *Lancet*, 1: 637-639, 1989.
36. Welt, K., Fitz, F., and Schaffranietz, L.: Ginkgo biloba extract (EGb 761) protects ultrastructure of hypoxic myocardium in elderly rats, in *Proceedings of the International Symposium on Natural Antioxidants. Molecular Mechanisms and Health Effects*, edited by Packer, L., Traber, M.G., and Xin, W., Champaign (IL), AOCS Press, 1995, pp. 424-433.
37. Sastre, J., Millan, A., de la Asuncion, J.G., Pallardo, F.V., Droy-Lefaix, M.T., and Vinal, J.: Prevention of age-associated mitochondrial DNA damage by Ginkgo biloba extract EGb 761, in *Proceedings of the VIII Biennial Meeting International Society for Free Radical Research*, Barcelona, J.R. Prous, (in press).
38. Spinnewyn, B., Blavet, N., and Drieu, K.: Effect of Ginkgo biloba extract (EGb 761) on oxygen consumption by isolated cerebral mitochondria, in *Advances in Ginkgo biloba Extract Research*, Vol. 4, Effects of Ginkgo biloba Extract (EGb 761) on Aging and Age-related Disorders, edited by Christen, Y., Courtois Y., and Droy-Lefaix, M.T., Paris, Elsevier, 1995, pp. 17-22.
39. Demopoulos, H., Flamm, E., Seligman, M., Power, R., Pietronigro, D., and Ransohoff, J.: Molecular pathology of lipids in CNS membranes, in *Oxygen and Physiological Function*, edited by Jöbsis, F.F., Dallas (TX), Professional Information Library, 1977, pp. 491-508.
40. Pastuszko, A., Gordon-Majozak, W., and Dabrowiecki, Z.: Dopamine uptake in striatal synaptosomes exposes to peroxidation "in vitro". *Biochem. Pharmacol.*, 32: 141-146, 1983.
41. Dumont, E., Petit, E., d'Arbigny, P., Tarrade, T., and Nouvelot A.: Ginkgo biloba extract (EGb 761) protects membrane polyunsaturated fatty acids and proteins against degradation induced by UV-C irradiation, in *Advances in Ginkgo biloba extract*, Vol. 2, Ginkgo biloba extract (EGb 761) as a free radical scavenger, edited by Ferradini, C., Droy-Lefaix, M.T., and Christen, Y., Paris, Elsevier, 1993, pp. 173-184.
42. Ramassamy, C., Christen, Y., Clostre, F., and Costentin, J.: The ginkgo biloba extract (EGb 761) increases synaptosomal uptake of 5 hydroxy tryptamine; in vitro and ex vivo studies. *J. Pharm. Pharmacol.*, 44: 943-945, 1992.
43. Huguet, F., Drieu, K., Tarrade, T., and Piriou, A.: Cerebral adrenergic and serotonergic receptor loss in rats during aging can be reversed by Ginkgo biloba extract (EGb 761), in *Advances in Ginkgo biloba extract research*, Vol. 4, Effects of Ginkgo biloba extract (EGb 761) on aging and age-related disorders, edited by Christen, Y., Courtois, Y., and Droy-Lefaix, M.T., Paris, Elsevier, 1995, pp. 77-88.
44. Heron, D.S., Schinitzky, M., Hershkowitz, M., and Samuel, D.: Lipid fluidity markedly modulates the binding of serotonin to mouse brain membranes. *Proc. Natl. Acad. Sci. USA*, 77: 7463-7467, 1980.
45. Tricklebank, M.P., Forler, C., Fozard, J.R.: The involvement of subtypes of the 5-HT₁ receptor and of catecholaminergic systems in the behavioural response to 8-hydroxy-2 (din-propylamino) tetralin in the rat. *Eur. J. Pharmacol.*, 106: 271-282.
46. Sram, R.J., Binkova, B., Stejskalova, J., and Topinka, J.: Effect of EGb 761 on lipid peroxidation, DNA repair and antioxidant activity, in *Advances in Ginkgo biloba Extract Research*, Vol. 2, Ginkgo biloba Extract (EGb 761) as a Free Radical Scavenger, edited by Ferradini, C., Droy-Lefaix, M.T., and Christen, Y., Paris, Elsevier, 1993, pp. 27-38.
47. Holgado-Madruga, M., de Castro, S., and Macias-Nunez, J.F.: Effects of Ginkgo biloba extract (EGb 761) on brain aging and oxygen free-radical metabolism in the rat, in *Advances in Ginkgo biloba Extract Research*, Vol. 4, Effects of Ginkgo biloba Extract (EGb 761) on Aging and Age-related Disorders, edited by Christen, Y., Courtois, Y., and Droy-Lefaix, M.T., Paris, Elsevier, 1995, pp. 71-76.

48. Halliwell, B.: Oxygen radicals as key mediators in neurological disease : fact or fiction. *Ann. Neurol.*, 32: 910-915, 1992.
49. Siesjö, B.K.: A new perspective of ischemic brain?, in *Neurobiology of Ischemic Brain Damage*, edited by Kogure, K., Hossmann, K.A., and Siesjö, B.K., Amsterdam, Elsevier, 1993, pp. 1-9.
50. Spinnewyn, B., Blavet, N., and Clostre, F.: Effets de l'extrait de Ginkgo biloba sur un modèle d'ischémie cérébrale chez la gerbille. *Presse. Med.*, 15: 1511-1515, 1986.
51. Lavin, M.F., Baxter, G.D., Song, Q., Findik, D., and Kovacs, E.: Protein modification in apoptosis, in *Programmed Cell Death, the Cellular and Molecular Biology of Apoptosis*, edited by Lavin, M., and Watters, D., Brisbane, Harwood Academic Pub., 1993, pp. 45-57.
52. Tomei, D.L., and Cope, F.O.: Apoptosis. The molecular basis of cell death. *Current communications in cell and molecular biology*, New York, Cold Spring Harbor Press, 1991.
53. Slater, A.F.G., and Orrenius, S.: Oxidative stress and apoptosis, in *Oxidative Stress and Aging*, edited by Cutler, R.G., Packer, L., Bertram, J., and Morri, A., Basel, Birkhäuser Verlag, 1995, pp. 21-26.
54. Selkoe, D.J.: The molecular pathology of Alzheimer's disease. *Neurons*, 6: 487-498, 1991.
55. Buttke, T.M., and Sandstrom, P.A.: Oxidative stress as a modulator of apoptosis. *Immunol. Today*, 15: 7-10, 1994.
56. Didier, A., Rouiller, D., Coronas, V., Jourdan, F., and Droy-Lefaix, M.T.: Effects of Ginkgo biloba extract (EGb 761) on apoptosis and regeneration of primary olfactory neurons following target "lesioning" in rats, in *Advances in Ginkgo biloba Extract Research*, Vol. 5, Effects of Ginkgo biloba Extract (EGb 761) on Neuronal Plasticity, edited by Christen, Y., Droy-Lefaix, M.T., and Macias-Nunez, J.F., Paris, Elsevier, 1996, pp. 45-52.
57. Del Maestro, R.F., Thaw, H.H., Bjork, J., Planker, M., and Arfors, K.E.: Free radicals as mediators of tissue injury. *Acta Physiol. Scand.*, 492: 43-57, 1980.
58. Szabo, M.E., Droy-Lefaix, M.T., Doly, M., and Braquet, P.: Free radical-mediated effects in reperfusion injury: an histologic study with superoxide dismutase and EGb 761 in rat retina. *Ophthalmic Res.*, 23: 225-234, 1991.
59. Szabo, M.E., Droy-Lefaix, M.T., Doly, M., Carré, C., and Braquet, P.: Ischemia and reperfusion-induced histologic changes in the rat retina. Demonstration of a free-radical mediated mechanism. *Invest. Ophthalmol. Vis. Sci.*, 32: 1471- 1478, 1991.
60. Szabo, M.E., Droy-Lefaix, M.T., Doly, M., and Braquet, P.: Modification of ischemia-reperfusion-induced ion shifts (Na⁺, K⁺, Ca²⁺, Mg²⁺). *Ophthalmic Res.*, 25: 1-9, 1993.
61. Menerath, J.M., Cluzel, J., Droy-Lefaix, M.T., and Doly, M.: Electroretinographic assessment of a free radical scavenger (EGb 761) treatment on rat retinal ischemia. *Invest. Ophthalmol. Vis. Sci.*, 36: S934, 1995.
62. Menerath, J.M., Doly, M., Droy-Lefaix, M.T., Besse, G., and Vennat, J.: Evaluation electroretinographique et histologique de l'ischémie rétinienne chez le rat par hypertonie intra-oculaire. *Ophthalmologie*, 9: 291-300, 1995.
63. Behar-Cohen, F.F., Heydolph, S., Faure, V., Droy-Lefaix, M.T., Courtois, Y., and Goureau, O.: Peroxynitrite cytotoxicity on bovine retinal pigmented epithelial cells in culture. *Biochem. Biophys. Res. Comm.*, 226: 842-849, 1996.
64. Droy-Lefaix, M.T., Bonhomme, B., and Doly, M.: A protective effect of EGb 761 on aging rat retina. *Invest. Ophthalmol. Vis. Sci.*, 36: S855, 1995.
65. Esterbauer, H., Gebicki, J., Puhl, H., and Jürgens, G.: The role of lipid peroxidation and antioxidants in oxidative modification of LDL. *Free Rad. Biol. Med.*, 13: 341-390, 1992.
66. Fortun, A., Khalil, A., Bonnefont-Rousselot, D., Gardès-Albert, M., Lepage, S., Delattre, J., and Droy-Lefaix, M.T.: Effect of EGb 761 on the peroxidation of human low-density lipoproteins (LDL) initiated by oxyradicals generated by water radiolysis. *J. Chim. Phys.*, 91: 1078-1084, 1994.
67. McCord, J.M.: Oxygen-derived free radicals in post ischemic tissue injury. *N. Engl. J. Med.*, 312: 159-163, 1985.
68. Blasig, I.E., Ebert, B., Hennig, C., Pali, T., and Tosaki, A.: Inverse relationship between ESR spin trapping of oxyradicals and degree of functional recovery during myocardial reperfusion in isolated working rat heart. *Cardiovasc. Res.*, 24: 263-270, 1990.
69. Terada, L.S., Rubinstein, J.D., Lesnefsky, E.J., Horwitz L.D., Left, J.A., and Repine, J.E.: Existence and participation of xanthine-oxidase in reperfusion injury of ischemic rabbit myocardium. *Am. J. Physiol.*, 260: H805-H810, 1991.
70. Packer, L., Haramaki, N., Kawabata, T., Marcocci, L., Maitra, I., Maguire, J.J., Droy-Lefaix, M.T., Sekaki, A.H., and Gardès-Albert, M.: Ginkgo biloba extract (EGb 761); antioxidant action and prevention of oxidative stress-induced injury, in *Advances in Ginkgo biloba Extract Research*, Vol. 4, Effects of Ginkgo biloba Extract (EGb 761) on Aging and Age-related Disorders, edited by Christen Y., Courtois, Y., and Droy-Lefaix, M.T., Paris, Elsevier, 1995, pp. 23-47.

71. Packer, L.: Oxidative stress, antioxidants aging and disease, in *Oxidative Stress and Aging*, edited by Cutler, R.G., Packer, L., Bertram, J., and Morri, A., Basel, Birkhäuser Verlag, 1995, pp. 1-14.
72. Culcasi, M., Pietri, S., Carriere, I., d'Arbigny, P., and Drieu, K.: Electron-spin-resonance study of the protective effects of Ginkgo biloba extract (EGb 761) on reperfusion-induced free radical generation associated with plasma ascorbate consumption during open-out surgery in man, in *Advances in Ginkgo biloba Extract Research*, Vol. 2, Ginkgo biloba Extract (EGb 761) as a Free Radical Scavenger, edited by Ferradini, C., Droy-Lefaix, M.T., and Christen, Y., Paris, Elsevier, 1993, pp. 153-162.
73. Taillandier, J., Ammar, A., Rabourdin, J.P., Ribeyre, J.P., Pichon, J., Niddam, S., and Pierart, H.: Ginkgo biloba extract in the treatment of cerebral disorders due to aging. Longitudinal, multicenter, double-blind study versus placebo, in *Rökan (Ginkgo biloba) Recent Results in Pharmacology and Clinic*, edited by Fünfgeld, E.W., Berlin, Springer-Verlag, 1988, pp. 291-301.
74. Allain, H., Lieury, A., Gandon, J.M., and d'Arbigny, P.: The dual-coding test in elderly subjects: psychometric assessment of properties of Ginkgo biloba extract (EGb 761), in *Advances in Ginkgo biloba Extract Research*, Vol. 4, Effects of Ginkgo biloba Extract (EGb 761) on Aging and Age-Related Disorders, edited by Christen, Y., Courtois, Y., and Droy-Lefaix, M.T., Paris, Elsevier, pp. 131-139.
75. Israël, L., Myslinski, M., Dell'Accio, E., Lambert, C., and Hugonot, R.: Onset of memory disorders: specific and combined contributions of memory-training programs and Ginkgo biloba extract (EGb 761) treatment, in *Advances in Ginkgo biloba Extract Research*, Vol. 4, Effects of Ginkgo biloba Extract (EGb 761) on Aging and Age-Related Disorders, edited by Christen, Y., Courtois, Y. and Droy-Lefaix, M.T., Paris, Elsevier, 1995, pp. 119-129.
76. Hofferberth, B.: Influence of Ginkgo biloba Extract (EGb 761) on neurophysiological and neuropsychological measurements in patients suffering from psychoorganic syndrome, in *Advances in Ginkgo biloba Extract Research*, Vol. 4, Effects of Ginkgo biloba Extract (EGb 761) on Aging and Age-Related Disorders, edited by Christen, Y., Courtois, Y., and Droy-Lefaix, M.T., Paris, Elsevier, 1995, pp. 141-148.
77. Kanowski, S.: Proof of efficacy of the Ginkgo biloba special extract (EGb 761) in outpatients suffering from primary degenerative dementia of the Alzheimer type and multi-infarct dementia., in *Advances in Ginkgo biloba Extract Research*, Vol. 4, Effects of Ginkgo biloba Extract (EGb 761) on Aging and Age-Related Disorders, edited by Christen, Y., Courtois, Y., and Droy-Lefaix, M.T., Paris, Elsevier, 1995, pp. 149-158.